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Research Article
Effects of Season, Species and Botanical Fraction on Oxalate Acid in *Brachiaria* Spp. Grasses in Yogyakarta, Indonesia

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Abstract

Background and Objective: Grasses have relatively few intrinsic toxins. Grasses that contain intrinsic toxins include tropical grasses (oxalate acid). This study examined the nutrient and oxalate acid content of *Brachiaria* in different species, seasons and botanical fractions in Indonesia. **Materials and Methods:** The species of *Brachiaria* used were *Brachiaria brizantha*, (*B. brizantha*) *Brachiaria decumbens* (*B. decumbens*), *Brachiaria ruziziensis* (*B. ruziziensis*) and *Brachiaria humidicola* (*B. humidicola*). The botanical fractions considered were the leaf and stem. The oxalate acid content was analyzed using High-Performance Liquid Chromatography (HPLC). The data were analyzed using analysis of variance. **Results:** It was observed that the oxalate acid content in the dry season was higher than that in the rainy season ($p<0.05$). The results showed that the leaves of all *Brachiaria* species had higher dry matter, crude protein and extract ether content than the stem in the dry season ($p<0.05$). **Conclusion:** *Brachiaria* grasses cut in the dry season had higher oxalate content than those cut in the rainy season.

Key words: Botanical fraction, oxalate acid, *Brachiaria* spp., season

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Brachiaria grass is used mostly as a pasture plant, has good production and grows well under marginal conditions of low soil fertility and Al toxicity[1]. *Brachiaria* grass comes from the African region (Uganda, Kenya, Tanzania) and has spread in the Asia and Pacific regions. It was introduced to Indonesia in 1958[2]. There are several species of *Brachiaria* grass, including *Brachiaria brizantha* (palisade grass), *Brachiaria decumbens* (signal grass), *Brachiaria humidicola* and *Brachiaria ruziziensis* (ruzi grass).

Grasses have relatively few intrinsic toxins. Grasses that contain intrinsic toxins include tropical grasses (oxalate and saponin). The effect of oxalate is bone demineralization. Oxalate reacts with calcium to produce insoluble calcium oxalate, reducing calcium absorption[3]. Oxalate acid has an anhydrous, crystallized from glacial acetic acid and being pyramidal or elongated octahedral. It is an odorless white solid. Molecular formula of oxalate acid is $C_2H_2O_4$. It is a relatively strong organic acid. Its name comes from the oxalis plant (reddish-brown wood sorrel), from which it was first isolated[4]. Oxalate acid has the ability to form strong bonds with various minerals, such as sodium, potassium, magnesium and calcium[5].

Oxalate acid is able to bind Ca in the gastrointestinal tract and blood to form insoluble Ca oxalate, carries in a small amount in the Ca serum levels and causes damage to the kidneys. The role of oxalate acid in plants includes calcium regulation, ion balance (Na and K), protecting plants, tissue support and heavy metal detoxification. The oxalate acid content in plants is influenced by season, species and botanical fraction. Soluble oxalate is readily absorbed in the systemic circulation and could react with blood Ca, causing hypocalcemia. Oxalate forms insoluble crystals of calcium oxalate, which inhibit the kidney tubules[6].

The oxalate acid content of alfalfa (*Medicago sativa*) has been reported to interfere with the utilization of Ca in sheep and poultry[7] and some ruminant and non-ruminant herbivore species. The high content of oxalate acid in plants could cause poisoning. For example, deaths in cows and buffaloes have occurred after they fed on elephant grass that contained high levels of oxalate acid (3.01%). Information regarding the levels of oxalate in *Brachiaria* is limited and increasing the knowledge base regarding the oxalate acid and nutrient content in these species could result in a more efficient use of this forage. Therefore, this study was undertaken to improve the information regarding seasonal and varietal effects, nutrient content and oxalate botanical fractions in various *Brachiaria* spp.

MATERIALS AND METHODS

This study was conducted in the field at the Pasture and Forage Science Laboratory, Faculty of Animal Science, Gadjah Mada University, Yogyakarta, during the period between September, 2015 (rainy season) and March, 2016 (dry season). This region, according to the Station of Meteorology, Climatology and Geophysics[8], has a monthly rainfall of 94.5-324 mm, air temperature of 20.9-33.3°C and humidity of 41-88% (Fig. 1). The chemical composition of the soil in this region is shown in Table 1.

Materials: The species of *Brachiaria* used were *B. brizantha*, *B. decumbens*, *B. ruziziensis* and *B. humidicola*.

Methodology

Sample collection and preparation: The *Brachiaria* spp. were harvested twice in the dry season and rainy season. The botanical fractions (leaf and stem) were observed. The harvested samples were chopped and dried at 55°C for 72 h. Then, the dried samples were grinded in a Wiley mill and sieved through a 1 mm screen.

Oxalate analysis: The content of oxalate acid was determined using High-Performance Liquid Chromatography (HPLC). Samples (0.5 g) were extracted with 15 mL of distilled water and 1 M HCl. The suspension of sample was heated in a bath of boiling water for 18 min. After cooling to room temperature, the mixture was filtered through filter paper (Type 5A, Toyo Roshi Kaisha Ltd., Japan), wash with water and made up to 50 mL and passed through a hydrophilic membrane filter (0.45 µL) prior to HPLC analysis. The analysis of oxalate was conducted on a column of Shodex IC SI-90 4E (size: 4,0 mmID) ×250 mmL, Shodex Group, Showa Denko K. K., Tokyo, Japan)

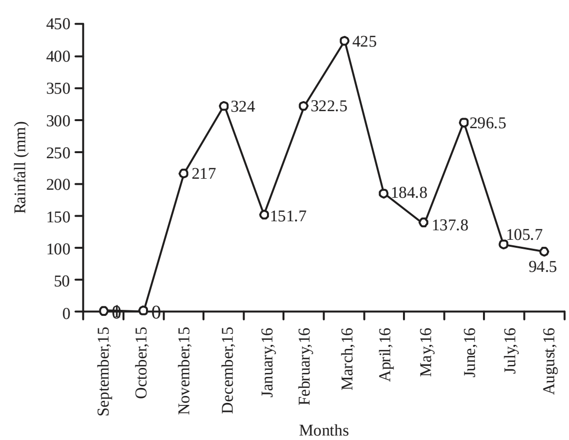

Fig. 1: Monthly rainfall (mm) in Yogyakarta, Indonesia

Table 1: Chemical composition of the soil from the experimental site

pH	C (%)	OM (%)	Total N (%)	Total P (%)	Total K (%)	C/N ratio
7.12	1.87	3.23	0.20	0.22	0.10	9.35

OM: Organic matter

Table 2: Nutrient value of leaves (%) from four *Brachiaria* spp. in differences seasons in Yogyakarta, Indonesia

		Seasons	
Species	Items	Dry	Rainy
Brachiaria brizantha	DM	33.46±1.78[b]	25.90±2.60[a]
	OM	88.54±1.18[ns]	86.90±0.75[ns]
	CF	29.53±1.35[ns]	28.48±0.62[ns]
	EE	4.50±0.50[b]	3.22±0.60[a]
	CP	8.05±0.51[a]	10.78±0.23[b]
Brachiaria ruziziensis	DM	25.52±1.31[b]	22.87±1.44[a]
	OM	88.86±1.80[ns]	87.28±0.55[ns]
	CF	25.59±2.40[a]	24.02±0.46[b]
	EE	4.70±0.24[b]	2.73±0.44[a]
	CP	9.67±0.44[a]	12.49±0.33[b]
Brachiaria decumbens	DM	25.01±1.09[b]	22.06±1.58[a]
	OM	87.72±1.49[ns]	86.77±3.52[ns]
	CF	28.92±2.07[ns]	26.38±2.18[ns]
	EE	4.13±0.24[b]	4.03±1.30[a]
	CP	8.80±1.55[a]	11.95±0.08[b]
Brachiaria humidicola	DM	31.08±1.34[b]	25.87±1.87[a]
	OM	89.00±1.65	88.04±0.84[ns]
	CF	30.34±10.88	23.43±1.83[ns]
	EE	4.43±0.81[b]	3.39±0.44[a]
	CP	7.83±0.29[a]	10.43±0.42[b]

[a,b]Different superscripts on the same line indicate a significant difference (p<0.05) [ns]Not significant, DM: Dry matter, OM: Organic matter, CF: Crude fiber, EE: Ether extract protein

Table 3: Nutrient value of stems (%) from four *Brachiaria* spp. in different seasons in Yogyakarta, Indonesia

		Seasons	
Species	Items	Dry	Rainy
Brachiaria brizantha	DM	30.95±1.62[b]	25.37±1.18[a]
	OM	87.74±0.82[ns]	84.68±4.42[ns]
	CF	28.44±4.39[ns]	26.01±1.20[ns]
	EE	4.16±0.05[b]	3.89±0.12[a]
	CP	2.93±0.08[a]	4.92±0.13[b]
Brachiaria ruziziensis	DM	30.65±1.35[b]	20.35±1.69[a]
	OM	88.25±0.35[ns]	87.10±0.77[ns]
	CF	32.88±2.54[ns]	30.56±2.70[ns]
	EE	4.10±0.21[b]	3.33±0.14[a]
	CP	5.51±0.26[a]	7.07±0.57[b]
Brachiaria decumbens	DM	28.56±1.14[b]	20.15±1.56[a]
	OM	88.22±0.75[ns]	86.62±1.26[ns]
	CF	30.09±1.02[ns]	27.72±3.30[ns]
	EE	4.58±0.12[b]	3.89±1.01[a]
	CP	3.98±0.03[a]	5.09±0.13[b]
Brachiaria humidicola	DM	30.51±1.32[b]	17.44±1.32[a]
	OM	89.19±1.45[ns]	86.78±0.20[ns]
	CF	30.73±1.43[ns]	28.61±8.72[ns]
	EE	4.55±0.57[b]	2.57±0.34[a]
	CP	3.60±0.20[a]	5.26±0.17[b]

[a,b]Different superscripts on the same line indicate a significant difference (P<0.05) [ns]Not significant, DM: Dry matter, OM: Organic matter, CF: Crude fiber, EE: Ether extract protein

equipped with a guard column IC SI-90G (4.6 mm×10 mmL) using a HPLC system (LC 10 A, Shimadzu Co. Ltd., Japan) composed of a pump (LC-10 ADVP), a system controller (SCL-10 AVP) and a column oven (CTO-10 AVP). A sample of 10 µL was chromatographed at 30°C using 15 mM $NaHCO_3$ as eluent at a flow rate of 1.5 mL min^{-1}. Oxalate was detected at 210 nm using diode array detector (SPD-M10 AVP). The amount of oxalate was determined with a reference to an authentic oxalate. The amount of insoluble oxalate was evaluated as the difference between the total and the soluble[9].

Statistical analysis: All the data were analyzed using analysis of variance and statistically significant differences between the oxalate acid and nutrient content of the leaf and stem were determined using Student's t-test. The differences between mean values and among variants were calculated using the least significant difference (LSD) method[10] at the p = 0.05 probability level.

RESULTS AND DISCUSSION

Nutrient values of *Brachiaria* spp.: The nutrient values of the botanical fractions from four species of *Brachiaria* in different seasons are shown in Table 2. Based on Table 2, the dry matter (DM), ether extract (EE), crude protein (CP) and crude fibre (CF) of *B. ruziziensis* leaves in different seasons were significantly different (p<0.05) and the same was observed for *B. brizantha*, *B. decumbens* and *B. humidicola* in all cases except the values of CF. The DM, EE and CP of *Brachiaria* spp. stems showed significant differences (p<0.05) (Table 3). The nutrient values in the dry season were higher than those in the rainy season.

Subagiyo and Kusmartono[11] suggested that the season affects the quality and quantity of grass production. Grass quality in different seasons could change the nutrient content of grasses. The DM values of the different species showed ranges of 25.37-33.46% (*B. brizantha*), 20.35-30.65% (*B. ruziziensis*), 20.15-28.56% (*B. decumbens*) and 17.44-31.08% (*B. humidicola*). Hartadi *et al.*[12] showed that *B. brizantha* had a DM value of 32%, *B. decumbens* had a value of 24%, *B. humidicola* had a value of 32.1%[13] and *B. ruziziensis* had a value of 18-20%[14]. The DM values in this study fluctuated. This fluctuation was thought to be related to photosynthesis and the age of the plant. A decreased amount of soil water caused the opening of stomata to reduce leaf evaporation[15]. Older plants had little water content and a higher cell wall proportion, so the value of DM became higher[16].

The values of EE in this study were not in accordance with the results of research conducted by Lubis[17], in which the value of EE in *B. brizantha* was 1.94%, nor were in accordance with the results of a study conducted by Hartadi *et al.*[12], in which the value for *B. brizantha* was 1.3% and for *B. decumbens* was 0.8%. This difference was thought to be due to the samples that were used for the proximate analysis having been taken from young plants. Usually, young plants have high EE content because the lipids are hydrolyzed by lipase to glycerol and fatty acids in the active cells. The fatty acids are used in the synthesis of phospholipids and glycolipids for the formation of organelles. Fatty acids are also present in the form of protective layer compounds in the epidermis of stems, leaves and fruit[18].

The chemical composition of the plants was determined by environmental factors. The CP values in *Brachiaria* spp. ranged from 2.93-12.49%. The CP value of *B. ruziziensis* was 11.6%, while that in *B. decumbens* stems was 6.2%[2] and that in *B. brizantha* was 6.6%[12]. The CP content fluctuated according to the season. The CP content in the rainy season was higher than that in the dry season and higher in leaf parts. This result was thought to have occurred because the leaf is the site of photosynthesis and respiration. Leaves have good quality because they have higher protein content than the stem[19]. Van Soest[20] was in agreement that the stem has lower quality than the leaves. Similarly, in Deinum[21], the protein content in the stem was lower than that in the leaf part and the stem had higher cell wall content. Protein showed the opposite relationship with CF and its content was affected by the age of the plant. In general, the protein content decreased as the age of the plant increased[22]. The nutrient value of forages is influenced by the ratio of leaves to stems. In this study, the CF content of *B. ruziziensis* leaves in the dry season was higher ($p<0.05$). This result was thought to have occurred because the samples were from young plants, which are not yet able to perform photosynthesis perfectly. Robinson[23] reported that when plants are photosynthesizing, most of the fatty acids are made in the chloroplasts but most of these products are delivered to the cytoplasm for processing and then returned to the chloroplast for further processing.

Effects of season, species and botanical fraction on oxalate acid: Table 4 shows that the differences between seasons were significant ($p<0.05$). In the dry season, the leaf oxalate acid content of *B. decumbens* was higher (3.45%) but in the rainy season, the oxalate acid content decreased to 0.70%. The higher oxalate acid values in the dry season suggest that the duration of solar irradiance affects the ambient temperature. The highest oxalate acid content occurs before the rainy season begins and the oxalate acid value can reach 6% or more[6]. The oxalate acid content in the tropics is higher than that in the subtropics. Alfalfa (*Medicago sativa*) has a higher oxalate acid content than that in tropical grasses[24]. In the early summer in the subtropics, leaf and stem tissues exhibit nearly equivalent levels of oxalate, whereas in the late summer and late autumn, the oxalate levels in the leaf tissue are greater. The oxalate content in the early summer was higher than that in the late autumn despite the fact that the dry weight percentages of the leaves in the early summer and late autumn were similar[25]. The leaf and stem tissues had almost the same oxalate acid content when harvested in the dry season[9]. Many environmental effects, including temperature, day length, hours of sunlight and flowering induction associated with seasonal change, may also change oxalate levels[26]. The oxalate content in the leaf was higher than that in the stem. A similar result was reported by Middleton and Barry[27].

Table 4: Effects of season, species and botanical fraction on the oxalate acid content (%) of *Brachiaria* spp. in Yogyakarta, Indonesia

Species	Botanical fraction	Seasons: Dry	Seasons: Rainy
Brachiaria brizantha	Leaf	0.69 ± 0.12^b	0.48 ± 0.00^a
	Stem	0.63 ± 0.14^b	0.36 ± 0.00^a
Brachiaria ruziziensis	Leaf	0.67 ± 0.01^b	0.57 ± 0.02^a
	Stem	0.56 ± 0.04^b	0.44 ± 0.02^a
Brachiaria decumbens	Leaf	1.45 ± 2.02^b	0.70 ± 0.03^a
	Stem	0.69 ± 0.04^b	0.49 ± 0.02^a
Brachiaria humidicola	Leaf	1.36 ± 0.28^b	1.13 ± 0.02^a
	Stem	0.72 ± 0.28^b	0.34 ± 0.01^a

[a,b]Different superscripts on the same line indicate a significant difference ($p<0.05$)

Based on the analysis of variance, the species differences were not significant ($p>0.05$). The oxalate acid content of the four species of *Brachiaria* differed. Table 4 shows that *B. decumbens* had higher oxalate acid content than *B. brizantha*, *B. ruziziensis* and *B. humidicola.* This result was not in agreement with the results of research conducted by Middleton and Barry[27] showing that the oxalate acid values of *B. decumbens*, *Panicum maximum* and *Digitaria decumbens* were lower (less than 2%).

Based on the analysis of variance, the botanical fractions were not significantly different ($p>0.05$). Table 4 shows that the leaf tissue had higher oxalate acid content than the stem tissue. This result is in agreement with published reports[26] showing that the leaf tissue of setaria (*Setaria sphacelata*) exhibited a higher oxalate acid value than the stem tissue. The oxalate acid value of the leaf was 4.4% and that of the stem was 2.8%. Similar results were also found in kikuyu grass (*Pennisetum clandestinum*)[28], showing that the leaf tissue of kikuyu grass had a oxalate acid value of 2.44% and that of the stem tissue was 0.98% and in elephant grass (*Pennisetum purpureum*)[9], showing that the leaf tissue had a higher oxalate acid value (2.78%) than the stem tissue (2.05%). This demonstrates that the leaf tissue contains higher oxalate acid content than the stem in almost all species of grass. This study suggests the potential for using both the leaf and stem parts of *Brachiaria* spp. in the rainy season but in the dry season, using the leaves can result in a higher nutrient quality of grass as feed, especially in terms of CP and EE. In addition, this study will help forage researchers and farmers to manage the defoliation of *Brachiaria* so that they can improve the quality of grass as feed. Thus, the cutting management of grasses can be done at the right time after obtaining clear information about the nutrient and anti-nutrient composition of grasses.

CONCLUSION

Based on this study, it can be concluded that different seasons, species and botanical fractions did not significantly affect the oxalate acid content. Many environmental effects, including temperature, day length, hours of sunlight and flowering induction associated with seasonal change, may also change oxalate levels. The nutrient values (DM, EE and CP) of *Brachiaria* spp. in Indonesia were significantly different.

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